

TITLE: *Pseudomonas* spp. AS THE RESPONSIBLE AGENT FOR BLUE DISCOLORATION SPOILAGE IN FRESH CHEESE (MINAS FRESCAL)

AUTHORS: RODRIGUES, R.S.; MACHADO, S.G.; CARVALHO, A.F.; NERO, L.A.

INSTITUTION: UNIVERSIDADE FEDERAL DE VIÇOSA, VIÇOSA, MG (AV. PETER HENRY ROLFS, S/N - CAMPUS UNIVERSITÁRIO, CEP 36570-900, VIÇOSA – MG, BRAZIL)

ABSTRACT:

Dairy products with a high moisture content are usually likely to be spoiled by microorganisms, such as those belonging to the genus *Pseudomonas*. Several species of this genus are capable of producing unwanted extracellular enzymes such as proteases, lipases, and lecithinases, as well as pigments, which can even be produced under cold storage. Blue discoloration by *Pseudomonas* spp. has been reported as frequent in fresh cheeses in different countries, requiring the characterization of the isolates responsible for this spoilage for effective control in dairy facilities. The identification of isolates causing this spoilage is usually carried out with phenotypic tests, but a detailed characterization must be performed considering genetic approaches. In order to investigate this issue, samples of Minas frescal cheese (n = 2) showing blue discoloration were subjected to investigation for *Pseudomonas* spp. using *Pseudomonas* agar base supplemented with cephaloridine, fucidin and ceftrimide (CFC), incubated at 25 ± 1 °C for 48 h. Isolates obtained were streaked onto potato dextrose agar (BDA) and plate count agar (PCA) and incubated at different temperatures (5, 14, 25, and 32 °C) for 168 h. Two blue pigment-producing isolates were selected for whole genome sequencing (WGS) by Illumina Miseq platform and draft genomes, assembled with Mira v.4.9.6 software, were used for identification. Finally, phylogenomic analyzes were performed using the algorithms: average nucleotide identity based on BLAST (ANIb), and genome-to-genome distance (GGDC) method; both using type strains belonging to the *Pseudomonas fluorescens* phylogenetic subgroup. In addition, the Type (Strain) Genome Server – TYGS – was used to complement the result. We obtained 257 presumptive isolates for *Pseudomonas*, 31 of which were capable of producing blue pigment; these isolates were able to produce the pigment after 24 h of incubation at 5, 14 and 25 °C and only isolates from one of the samples were able to produce pigment at 32 °C after 4 d of incubation. ANIb and GGDC were able to identify isolates such as *Pseudomonas carnis* and *Pseudomonas paracarnis*, and with the use of TYGS it was possible to reproduce the same result. Therefore, we found that *P. carnis* and *P. paracarnis* are responsible for the spoilage of the analyzed Minas frescal cheeses, being capable of producing a blue pigment at low temperatures.

Keywords: spoilage, Minas frescal cheese, *Pseudomonas* spp., genomics

Development Agency: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil)